THE CELL LINEAGE OF PODARKE OBSCURA.

PRELIMINARY COMMUNICATION.

A. L. TREADWELL.

THE segmentation of Podarke is of especial interest as a representative of the "equal type" of cleavage among annelids. By the first three cleavages the ovum is divided into eight cells of equal size, four above and four below. The next division establishes inequalities in size among the cells, but until the 56-cell stage is reached the quadrants are exactly alike. At the 56-cell stage one quadrant becomes different from the other three, and soon after bilateral divisions begin.

The transition from the 2 to the 4-cell stage is accompanied by a rotation to the left, and the familiar cross furrows appear. The furrow at the top is at right angles to that at the bottom and much shorter. The furrow at the bottom has the same direction that it has in Nereis,[1] Amphitrite,[2] Arenicola,[3] and Crepidula,[4] and retains this direction until it is possible to distinguish the quadrants by means of other landmarks. Later, owing probably to variations in position of the cells forming it, the direction of this furrow may vary. The first two planes of cleavage have the same relation to the median axis of the embryo that they have in Amphitrite and Arenicola.

The second group of micromeres[5] are given off in a left-handed spiral. These cells arise at the same time and are equal in size, there being no large d^2. Simultaneously with the origin of these, the cells $a^{1.1}$, $b^{1.1}$, $c^{1.1}$, $d^{1.1}$ are formed at the upper pole.

In the transition from sixteen to thirty-two cells all the divi-

[1] E. B. Wilson, "Cell Lineage of Nereis," *Journ. of Morph.*, vol. vi.

[2] A. D. Mead, "Early Development of Marine Annelids," *Journ. of Morph.*, vol. xiii.

[3] C. M. Child, *Zoöl. Bull.*, vol. i, no. 2.

[4] E. G. Conklin, "Embryology of Crepidula," *Journ. of Morph.*, vol. xiii.

[5] The term "micromeres" is used here simply for convenience, the division of the "macromeres" being approximately equal during the first four divisions.

sions are dexiotropic. The third group of micromeres appears, and at the same time cells $a^{1.2}$, $b^{1.2}$, $c^{1.2}$, $d^{1.2}$ are formed; $a^{1.1}$, $b^{1.1}$, $c^{1.1}$, $d^{1.1}$ then divide to form the *primary trochoblasts*, and a little later the 32-cell stage is completed by the division of the second group of micromeres.

From thirty-two to sixty-four cells all divisions are laeotropic, and this period may be divided into three stages.

First, from thirty-two to forty cells. This is accomplished by the division of A_3, B_3, C_3, D_3, to form the fourth group of micromeres, and the formation of the apical rosette by the division of $a^{1.2}$, $b^{1.2}$, $c^{1.2}$, and $d^{1.2}$.

The micromeres of this fourth quartette are equal in size, and lie between the cell from which they arose and the left-hand descendant of the second group of micromeres of the corresponding quadrant. At this stage, since the cells given off from the four cells at the top have been smaller than the micromeres of the second and third generations, and since the apical rosette cells are very much smaller than the cells of the fourth generation of micromeres, it follows that the four cells surrounding the rosette are much the largest in the embryo. These cells later divide and form the prominent cross. (See Fig. 4.) The rosette cells elongate and push into the segmentation cavity, retaining their connection with the outside by only a slender stalk. They later come to the surface and divide. (See Fig. 3, where is shown also the relative size of cells at the two poles of the embryo; those at the upper pole are dividing to form the cross.)

Second, forty to fifty-six cells. This is accomplished by a division of cells $a^{1.1.1}$, $b^{1.1.1}$, $c^{1.1.1}$, $d^{1.1.1}$; $a^{1.1.2}$, $b^{1.1.2}$, $c^{1.1.2}$, $d^{1.1.2}$; $a^{1.2}$, $b^{1.2}$, $c^{1.2}$, $d^{1.2}$; and a^3, b^3, c^3, d^3. The first two of these sets of cells are the *primary trochoblasts*. By their division are formed sixteen cells, *all* of which acquire cilia and become the primary prototroch, which here, as in other forms, is composed of four distinct areas of ciliated cells. These later, by the addition of cells from the lower hemisphere, become united into a single band with a dorsal interruption. Before the completion of the prototroch band a strong tuft of cilia is formed at the apical pole.

Third. The 64-cell stage is completed by the division of $a^{2.1}$, $a^{2.2}$, $b^{2.1}$, $b^{2.2}$, $c^{2.1}$, $c^{2.2}$, $d^{2.1}$, $d^{2.2}$; and here arises the first distinction which I have discovered between the quadrants. While in three quadrants the division is such that the upper left-hand cell ($a^{2.2.1}$, etc.) is smaller than the one below it ($a^{2.2.2}$, etc.), in one quadrant the *lower* cell is much the smaller, has a peculiar deeply staining nucleus, and is easily distinguished from the corresponding cells in the other quadrants. (See Figs. 1 and 2, as also Fig. 5, where the size of this cell as compared with the corresponding one in the other quadrants is shown. It should be remembered that the "left-

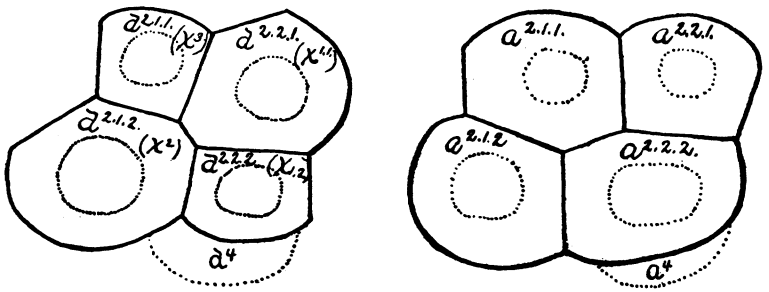

FIG. 1.—Second group of micromeres in D quadrant after their second division (64-cell stage).
FIG. 2.—Second group of micromeres of A quadrant at 64-cell stage. Compare relative sizes of $d^{2.2.2}$ and $a^{2.2.2}$.

hand" cell is the one which is on the left when looked at from the animal pole.) This lies immediately over one of the fourth group of micromeres, and a little to one side of the second cleavage line. For reasons which will appear more fully later on, I believe that this corresponds to the cell $x^{1.2}$, described in Amphitrite, Nereis, Arenicola, Crepidula, and Unio.[1]

The next divisions are those of cells $a^{1.3}$, $b^{1.3}$, $c^{1.3}$, $d^{1.3}$, at the upper pole, leading to the formation of the apical cross. Two of these cells divide equally or nearly so, while the other two divide very unequally. (See Fig. 4.) Here, although the divisions are still of the spiral type, a bilateral arrangement of cells results. The second line of cleavage passes in the direction indicated by the numerals. It is interesting to note that

[1] Lillie, "Embryology of the Unionidae," *Journ. of Morph.*, vol. x.

this division is dexiotropic, the form which it ought to assume under the law of alternating cleavages. A distinction between the anterior and posterior arms can be recognized during several divisions of the cross cells and until meridional divisions of its cells destroy the outline of the cross.

At the vegetative pole the next division is a bilateral one, of one of the fourth group of micromeres, accompanied by a bilateral division of the lower members of the third group which lie on either side of it. (See Fig. 5.) Here $c^{3.2}$ and $d^{3.2}$ have divided, while d^4 is still in process of karyokinesis. Just

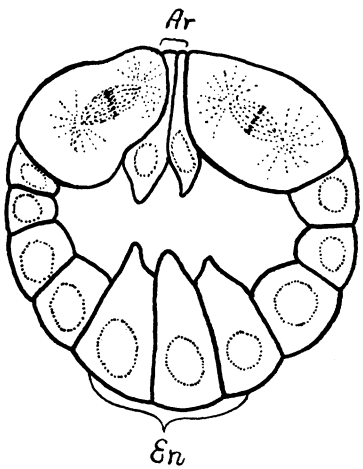

FIG. 3. — Optical section of embryo at the first division after the 64-cell stage. *Ar.*, apical rosette. *En.*, entoderm.

above this cell is the small cell $x^{1.2}$, and a plane passing through these cells coincides with the plane of bilateral symmetry of the cross.

This cell of the fourth quartette is $d^4 = M$; and, while the other three members of the fourth quartette migrate bodily into the segmentation cavity, its products remain at the surface until the blastopore has nearly closed. They then divide, each sending a cell into the segmentation cavity, and form the mesoblast bands.

The inner ends of the entoderm cells A_4, B_4, C_4, D_4 elongate very considerably, and the nuclei migrate to a considerable distance toward their inner end. (See Fig. 3, which shows the

beginning of the process.) Subsequently, however, these nuclei come to the surface and divide, forming the fifth group of micromeres. The eight cells thus formed invaginate, forming with the three members of the fourth quartette an invaginating plate of eleven cells, and the blastopore closes rapidly, closure being effected largely, if not entirely, by the division of the descendants of the third group of micromeres.

For purposes of comparison I have made a few observations on other forms with equal cleavage.

Lepidonotus sp.[1]

In Lepidonotus the bilateral cross and the small cell ($x^{1.2}$) appear exactly as in Podarke. These structures were overlooked by Mead,[2] who was also misled, by the appearance of

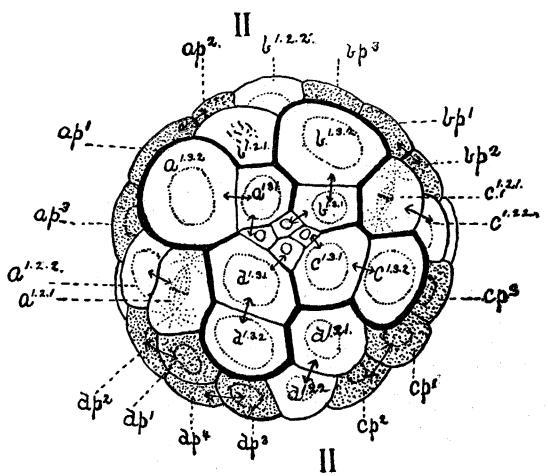

FIG. 4. — Cross when first formed, seen from animal pole. Some of the intermediate cells are dividing. Sixty-eight cells.

a "pseudo-invagination" similar to that of Podarke, into the statement that no fifth group of micromeres are formed. Here, as in Podarke, the nuclei migrate to the surface, and the micro-

[1] The most extended observations previously published on equal cleavage in annelid eggs are those of Mead on Lepidonotus. To Dr. Mead's generosity I am indebted for preparations of some of the later segmentation stages.

[2] *L. c.*, p. 267.

meres of the fifth generation are given off. These differ from other forms in the fact that the resulting micromeres a^5, b^5, c^5, d^5 are larger than the cells at the vegetative pole A_5, B_5, C_5, D_5.

Owing to lack of fresh material I have been unable to follow the history of the cross furrow, but it is interesting to

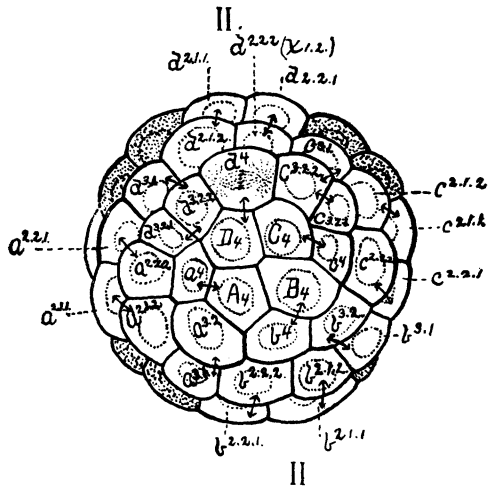

FIG. 5. — Seventy-cell stage from vegetative pole, showing bilateral division of d^4. $c^{3.2}$ and $d^{3.2}$ have already divided. The other three members of the fourth quartette are beginning to invaginate.

note that in all specimens which I have seen it has the same direction with respect to the cell $x^{1.2}$ which it has in Podarke.

I have been unable with the material at hand to discover the formation of the mesoderm.

Sthenolais picta.

I have followed the segmentation of this annelid only as far as the 64-cell stage, but in all respects it agrees exactly with Podarke. The cross furrow takes and retains the typical direction, and the cell $x^{1.2}$ is formed at the same place and time as in the other species.

Hydroides dianthus.

In this annelid the 32-cell stage is formed as in the other cases, but beyond this stage important differences appear. The

cross furrow seems not to retain its original position, and, although the small cell $x^{1.2}$ appears, other divisions of the second group of micromeres occur before any other landmarks are to be seen, and orientation is difficult. A fifth group of micromeres is given off, and the regular invaginating plate of eleven cells appears. One member of the fourth group of micromeres divides bilaterally at the surface, forming what I suppose is the mesoblast. The rosette is formed as in other cases, but the cross remains radially symmetrical until a late stage in the segmentation.

The primary trochoblasts arise as in Podarke, but do not divide again, thus forming two in each quadrant instead of four. Wilson [1] showed that the prototroch of Hydroides is composed of eight cells.

From these observations it is evident that equal cleavage in annelids is not in any way caused by or an expression of a lack of differentiation in the ovum. Although the dorsal cannot be distinguished from the ventral quadrant until the 64-cell stage, it is perfectly possible at the 4-cell stage to determine by means of the cross furrow the median plane of the future embryo. That this cross furrow sometimes varies in direction in later stages is fully explained by the fact that the cells forming it are very small (after the fourth group of micromeres are given off), and more or less of what I have called "pseudo-invagination" occurs. The furrow itself is short as compared with such forms as Nereis or Amphitrite. Under these conditions, as Conklin [4] has shown, the direction of the cross furrow may easily vary because of variations in pressure on the entoderm cells.

Further, the regular alternation from a right to a left-handed cleavage, the regular appearance of certain definite cells at certain definite times, indicate that we have here a differentiation as complete as in any form with unequal cleavage. At the 32-cell stage the embryo is approximately spherical, and is surrounded by a thin, much-wrinkled egg membrane. The four cells at the animal pole are a very little larger than those

[1] E. B. Wilson, *l. c.*, p. 398.

of the vegetative, but *are hard to distinguish from them except for the presence of the polar globules.* The next division, however, leads to the formation of the rosette cells at the top, and the fourth group of micromeres at the bottom, the latter division being nearly an equal one, the former very unequal.[1] It is difficult to explain cases of this kind except on the assumption of a complex differentiation in the protoplasm of the egg.

That mechanical conditions can play but a small part in the regulation of cell divisions is shown especially well by the small cell $x^{1.2}$. This cell arises in exactly the same manner in Amphitrite, Clymenella, Arenicola, Unio, and Crepidula (Conklin's $2d^{2.2}$) of the unequal, and in Lepidonotus, Sthenolais, Podarke, and Hydroides of the equal type. Comparison of the figures of the first forms with those of the second will show that mechanical conditions must be very different in the two cases. On the one hand, we have large mesoderm and entoderm cells which must exert considerable pressure on the cells surrounding them; on the other, the largest cells in the embryo are at the animal pole. These facts point to some definite complex organization of the egg protoplasm.

The problem to be settled by a study of equal cleavage has been stated by Mead thus:[2] "Whether one of the two cells in equal cleavage is homologous with the larger cell in unequal cleavage." I believe that one of these cells in the one case is homologous with one in the other, and that the second of Mead's alternatives is correct, that the "peculiar destiny of the cell" is "the cause of its larger size," and I would suggest that the cell *D* in the unequal type is larger, not simply because it contains somatic and mesodermic material, as Wilson supposed, but because it contains *an extra supply* of this material. Sufficient data for wide generalizations are not at hand, but such as we have bear out this supposition.

The trochophore of Podarke is small, with very thin walls and a feeble development of mesodermal tissue. It grows very slowly, so that scarcely any change except a slight increase in length is perceptible from twenty-four to seventy-two hours. Dr. Mead informs me that the same is true of the trochophore

[1] *Cf.* Mead, *l. c.*, p. 293. [2] *L. c.*, p. 278.

of Lepidonotus. Amphitrite, on the other hand, rapidly develops strong parapodia with numerous setae, and by sixty hours has four trunk segments. Compare also the four-day trochophore of Eupomatus [1] (Hatschek's Fig. 50, which strongly resembles the Podarke trochophore) with the sixty-hour trochophore of Nereis (Wilson's Fig. 91). The former is thin-walled with a large cavity and no trace of metameric segments or of parapodia. The latter has three pairs of segments, with large parapodia, setae, and cirri. If the law that the size of a cell bears some definite relation to the size and time of appearance of the organ to which it gives rise [2] applies in other cases, may it not apply here as well, and may we not suppose the extra amount of material stored in cell *D* of Nereis, Amphitrite, etc., is in some way related to the need for an extra amount of somatic and mesoblastic material in the young larva?

I think that the supposition is a reasonable one, and would state my conclusions thus. The large size of the posterior macromere *D* in forms with unequal cleavage is due to the fact that the young larvae of these forms require for their development an excessive amount of the characteristic products of this cell, — mesoblastic and somatic tissue, — and hence arises an accumulation of this material in this particular cell. This material differs, not in quality, but in quantity from that in the corresponding cell in equal cleavage (hence there is as truly a "precocious segregation" in the one case as in the other), and the two cells are to be regarded as absolutely homologous.

MARINE BIOLOGICAL LABORATORY,
WOODS HOLL, MASS.,
Aug. 23, 1897.

[1] Hatschek, "Entwick. d. Trochophore von Eupomatus." Wien, 1885.
[2] *Cf.* Lillie, *l. c.*, and Conklin, *l. c.*